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Synthesis of potent antitumor and antiviral benzofuran derivatives

Shadia A. Galal [a,*], Amira S. Abd El-All [a], Mohamed M. Abdallah [b], Hoda I. El-Diwani [a]

[a] Department of Chemistry of Natural and Microbial Products, Division of Pharmaceutical and Drug Industries Research, National Research Centre, Cairo, Egypt
[b] Univet Pharmaceuticals Ltd, Balteem, Egypt

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ABSTRACT

A new series of potent antitumor and antiviral benzofuran derivatives was synthesized by the reaction of the furochromone-6-carboxaldehydes **1** and **2** with different heterocyclic amines to yield the benzofuran-5-carbonyl derivatives **4–11**. The synthesized compounds **1**, **3–11** were tested against twelve different human cancer cell lines and all of the compounds were more potent than the comparative standards. The HIV inhibitory activity of the tested compounds **1**, **3–11** showed that they have higher potency than **Atevirdine**. Moreover, compound **6** was significantly potent with wider therapeutic index. The HIV-1 RT inhibitory activity showed that compounds **10**, **11**, **3** and **4** were notably potent but with lower therapeutic index than **Atevirdine**. The HCV NS3-4A protease inhibitor activity of the tested compounds revealed that they have weaker potency and less therapeutic index than **VX-950**, although compounds **1**, **4**, **9** and **6**, respectively exhibited significant activity.

Of the various human diseases, cancer, human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are scourges on humanity. Therefore, identification of novel potent, selective, and less toxic anticancer and antiviral agents remains one of the most pressing health problems. Benzofurans and furochromones[1–3] are very interesting heterocycles, which are ubiquitous in nature and show a wide range of biological activities. Recently, the anticancer and antiviral activities of the natural cyclopenta[*b*]benzofurans[4] and furochromones[5] have been reported, the same as for a variety of benzofuran derivatives. For example, 1-[(benzofuran-2-yl)phenylmethyl]imidazoles (**I**) had a potent, reversible, non-selective aromatase-inhibitory effect[6] and 2-{4-[(benzofuran-2-yl)carbonyl]piprazin-1-yl}-3-propylpyridine (**II**) exhibited good anti-HIV activity.[7] In the process of new drug discovery, benzofuran derivatives **III** and **IV** were obtained by screening for anticancer agents displaying selective cytotoxicity against a tumorigenic cell line (Fig. 1).[8–10]

On the other hand, pyrimidine nucleus is the main constituent of different antiviral and antitumor agents.[11–13] Several promising antitumor active derivatives were found to contain the fused benzimidazole and pyrimidine system as a series of 3,4-dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidine derivatives (**V**, **VI**).[14] Furthermore, compounds derived from fused pyridines and pyrimidines are useful for treating cell proliferation[15] and viral infections conditions (Fig. 2).[16]

The main purpose of this work consists of designing and synthesizing novel polycyclic compounds containing the benzofuran scaffold linked by a carbonyl group in the 5-position to the pharmacologically interesting moieties having pyrimidine nucleus and studying the influence of these moieties on antitumor, anti-HIV and anti-HCV activities of the synthesized compounds.

The naturally occurring furochromones namely as khellin **(VIIa)** and visnagin **(VIIb)** are very sensitive to alkali and the solvent used in the reaction has great effect on the obtained products, for example, aqueous alkaline hydrolysis of **VIIa** and **VIIb** using potassium hydroxide afforded khellinone **(VIIIa)** and visnaginone **(VIIIb)**, respectively.[17] Whereas, alcoholic hydrolysis of **VIIa** and **VIIb** with potassium hydroxide gave different product known as ω-acetokhellinone **(IXa)** and ω-acetovisnaginone **(IXb)**.[18] The hydrolysis products are important molecules for the synthesis of new furochromones. Compounds **VIIIa** and **VIIIb** are used in the synthesis of 5-oxo-5*H*-furo[3,2-g]chromene-6-carbaldehydes (**1**) and (**2**) directly via Vielsmeier-Haack reaction (c.f. Scheme 1).[19,20] The hydrolysis of chromones, known as ring opening reaction or γ-pyrone ring fission, was previously investigated by *ab initio* and density functional theory methods by Kóňa et al.[21] The calculations of ROR mechanism of khellin **VIIa** have been performed recently by Ati et al.[22]

An efficient way for synthesis of new benzofuran derivatives has been developed in this work, based upon ROR of chromones

* Corresponding author. Tel.: +20 233371718; fax: +20 233370931.
E-mail address: sh12galal@yahoo.com (S.A. Galal).

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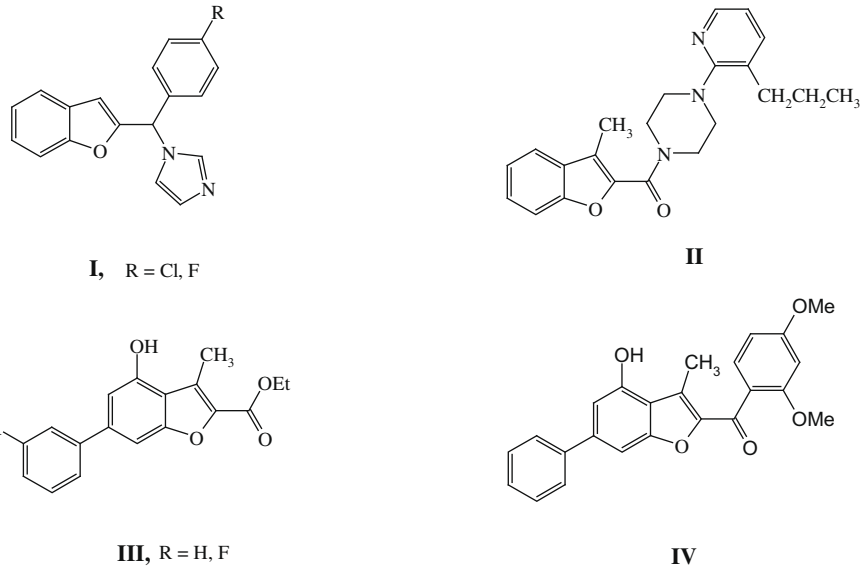


Figure 1.

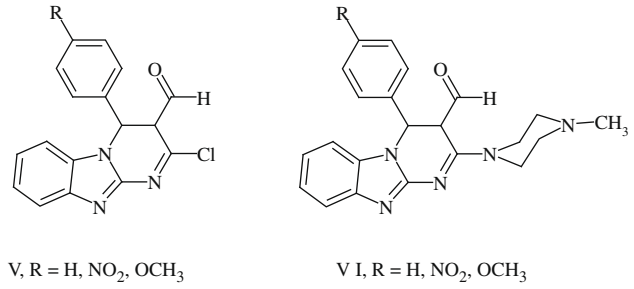


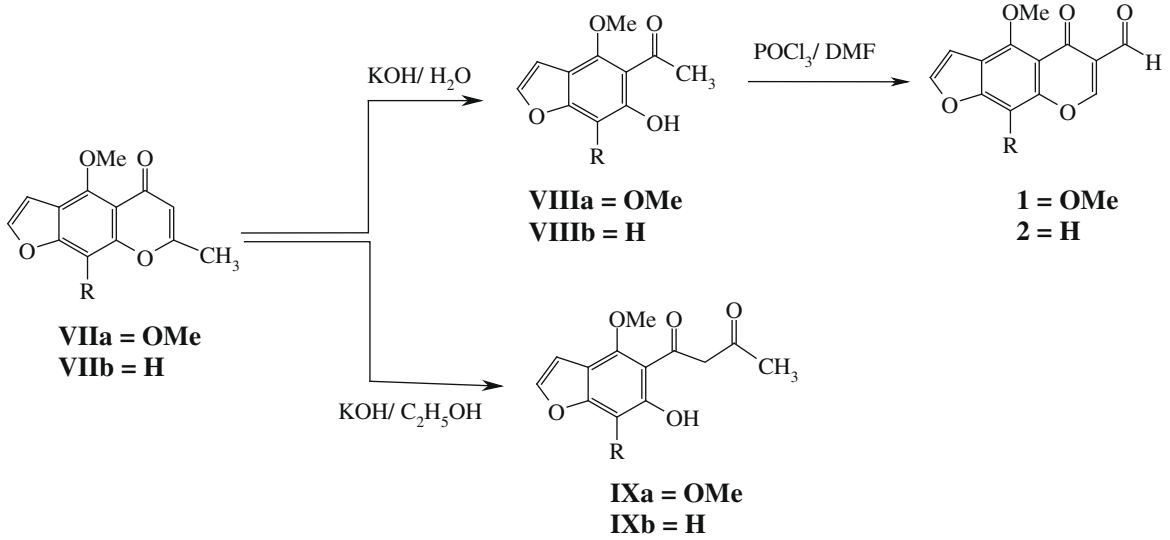
Figure 2.

in alkaline medium and the reaction of furochromones (**1** or **2**) with heterocyclic amines in mild basic conditions.

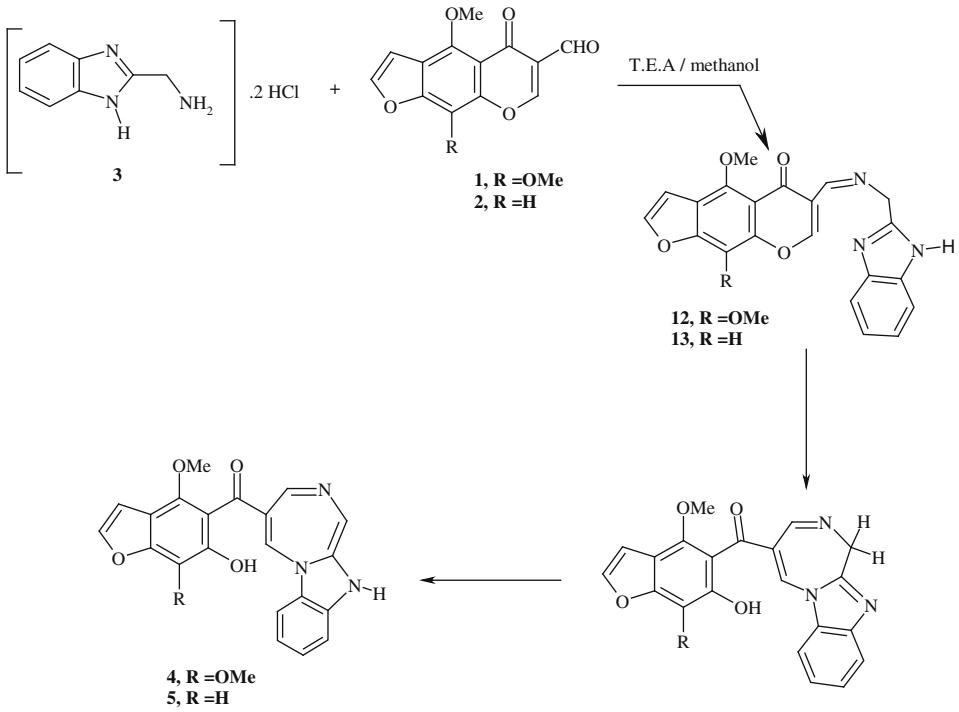
The reaction of (1*H*-benzimidazol-2-yl) methanamine dihydochloride (**3**)[23] with compounds **1** and **2** was studied by using a catalytic amount of triethyl amine or piperidine to give unexpected benzimidazo[1,2-a][1,4]diazepinyl derivatives **4** and **5**, respectively, characterized by the spectral and elemental analyses. The ^{1}H NMR spectrum of compound **4** indicated the absence of the (CH_2) singlet as well as the presence of two singlets at δ 9.25 and 12.75 ppm corresponding to NH and OH, which disappeared by D_2O. Also, the IR spectrum showed the presence of two bands at 3504 and 3415 cm^{-1} of the phenolic OH and NH of benzimidazole, respectively, which confirming the unexpected pyrone ring opening, although the pyrone ring was known to be stable enough in the presence of mild basic condition.[24]

Compound **4** or **5** was formed probably via the formation of the Schiff base **12** or **13** followed by a nucleophilic attack of the benzimidazole nitrogen at C-7 of compound **1** or **2** to cause pyrone ring opening and intramolecular cyclization affording **4** or **5**, respectively (c.f. Scheme 2).

Compounds **6–11** were obtained by the reaction of furochromone **1** with the following heterocyclic amines, 2-aminobenzimidazole, 2-aminopyridine, 2-aminopyrazine, 2-aminothiazole, 3-amino-5-methyloxazole, and 4-aminopyridine, respectively, in the presence of alcoholic KOH (c.f. Scheme 3).



Scheme 1.



Scheme 2.

The synthesis of compounds **6–11** probably took place via addition of hydroxyl ion to C^6–C^7 bond of furochromone derivative **1**[21,22] followed by a nucleophilic attack of the reacted amine on the intermediate **A**, formed by alcoholic hydrolysis of compound **1**, and finally, in the case of compounds **6–10**, the intramolecular cyclization took place (c.f. Scheme 4). Whereas, in the synthesizing of compound **11**, the intramolecular cyclization could not be occurred, the suggested mechanism of the reaction to afford **11** is explained in Scheme 4.

The cytotoxicity of the tested compounds **1**, **3–11** was determined by using MTT assay according to Mosmann's method[25] on different human cancer cell lines (c.f. Tables 1 and 2), including: cervical carcinoma (**KB**), ovarial carcinoma (**SKOV-3**), CNS cancer (**SF-268**), non-small lung cancer (**NCI H460**), colonadenocarcinoma (**RKOP 27**), anti-leukemia (**HL60**, **U937**, **K562**), melanoma (**G361**, **SK-MEL-28**), neuroblastoma (**GOTO**, **NB-1**). The cytotoxic effect of the tested compounds over normal human fetal lung fibroblast **WI 38** cell line was also tested. The results were expressed as the IC_{50}, which is the concentration of the drugs inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells.

Screening the cytotoxicity of the tested compounds on cervical carcinoma (KB), where **Fluorouracil** was used as standard drug ($IC_{50} = 4.46 \times 10^{-3}$ μM), showed that the tested compounds were more potent than the standard, with exception of compound **7** ($IC_{50} = 3.28 \times 10^{-3}$ μM) which had comparative activity with **Fluorouracil**. Compounds **4** and **5** were the most potent compounds ($IC_{50} = 2.55 \times 10^{-5}$ μM) and more potent than compounds **1** ($IC_{50} = 3.78 \times 10^{-5}$ μM) and **3** ($IC_{50} = 2.77 \times 10^{-5}$ μM).

On ovarian carcinoma (**SKOV-3**) cell line, compounds **4**, **5** and **10** were the most significantly potent compounds ($IC_{50} = 2.55 \times 10^{-5}$ μM) and more potent than both **Doxorubicin** used as standard drug ($IC_{50} = 4.16 \times 10^{-3}$ μM) and compound **1** ($IC_{50} = 3.28 \times 10^{-5}$ μM). Compounds **6** and **9** with ($IC_{50} = 3.28 \times 10^{-3}$ μM) showed less activity than compound **1** but still have more activity than **Doxorubicin**.

Studying the cytotoxicity of tested compounds on CNS cancer (**SF-268**) cell line, showed that **Cytarabine** ($IC_{50} = 7.68 \times 10^{-3}$ μM), the standard drug used, was less active than the tested compounds, which had comparative activity with $IC_{50} = 2.55 \times 10^{-5}$ μM to 3.00×10^{-5} μM.

On non-small lung cancer (**NCI H460**) and colonadenocarcinoma (**RKOP 27**) cell lines, all the tested compounds were more potent than **Gencitabine hydrochloride** ($IC_{50} = 2.13 \times 10^{-3}$ μM) and **Capecitabine** ($IC_{50} = 4.33 \times 10^{-3}$ μM), the standard drugs used, respectively.

The study of the cytotoxicity on leukemia (**HL60**) cell line indicated that, compounds **4** ($IC_{50} = 3.25 \times 10^{-5}$ μM) and **1** ($IC_{50} = 3.13 \times 10^{-5}$ μM) were the least potent compounds but more active than **Doxorubicin** ($IC_{50} = 1.13 \times 10^{-3}$ μM).

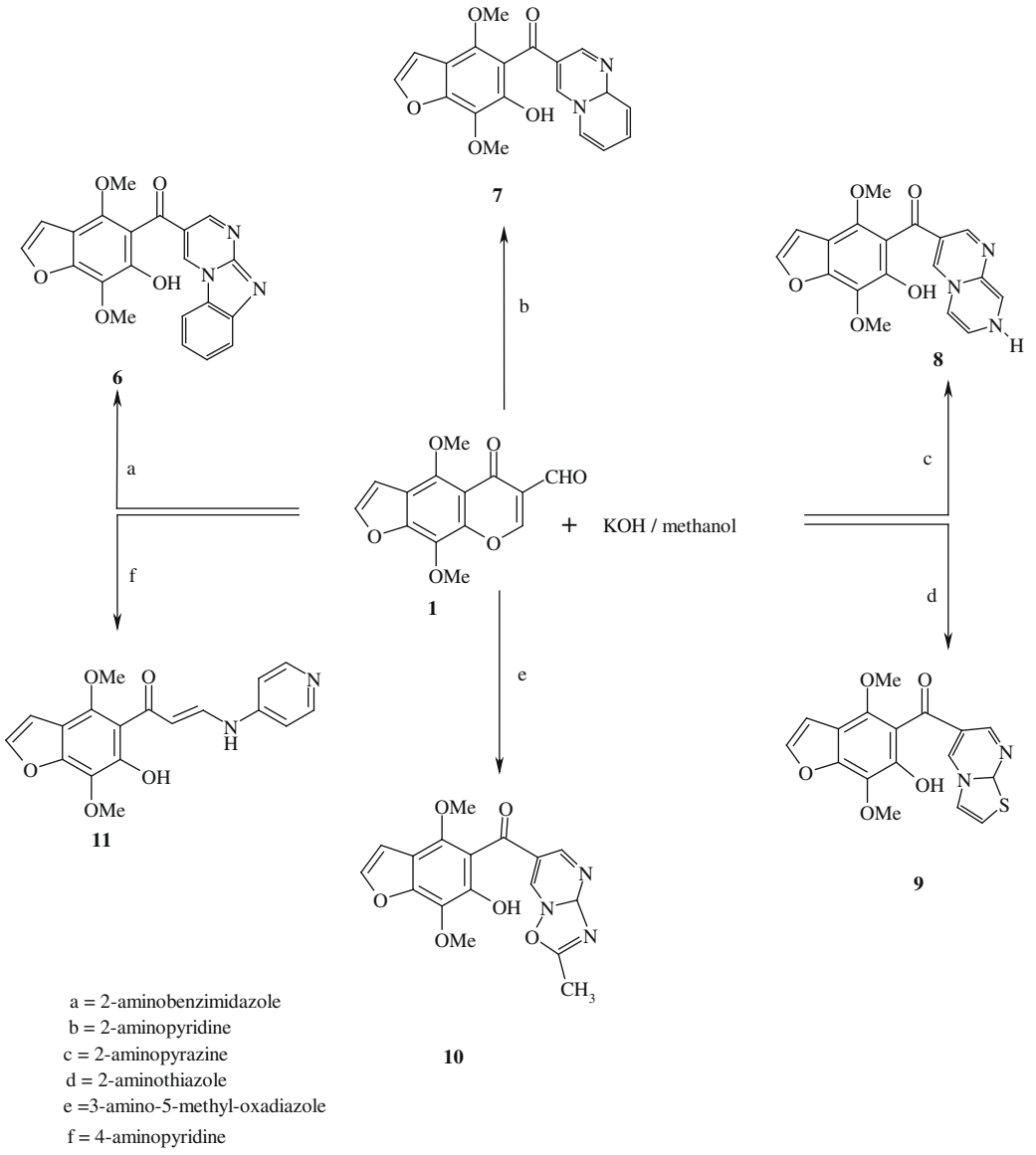
On leukemia **(U937)** cell line, compound **11** ($IC_{50} = 2.63 \times 10^{-5}$ μM) was the most potent, whereas, **4** showed the least bioactivity ($IC_{50} = 3.33 \times 10^{-5}$ μM), but with higher bioactivity than **Doxorubicin** ($IC_{50} = 4.45 \times 10^{-3}$ μM).

On leukemia **(K562)** cell line, compound **1** ($IC_{50} = 2.58 \times 10^{-5}$ μM) was the most potent among the other synthesized benzofuran derivatives, followed by compound **11** with ($IC_{50} = 2.63 \times 10^{-5}$ μM), whereas compound **9** was the least potent one with ($IC_{50} = 4.64 \times 10^{-5}$ μM), but was more potent than **Doxorubicin** ($IC_{50} = 6.66 \times 10^{-3}$ μM).

Estimation of the cytotoxicity on melanoma **(G361)** cell line showed that compounds **1**, **6** and **11** have the lowest IC_{50} (2.63×10^{-5} μM) among the tested compounds, whereas compound **7** showed the highest one ($IC_{50} = 4.81 \times 10^{-5}$ μM). On the other hand, comparing the IC_{50} of compound **7** to that of **Aldesleukin** (6.66×10^{-3} μM), the standard drug used, indicated that compound **7** was potent with over than 100 times.

On melanoma **(SK-MEL-28)** cell line, **Aldesleukin** ($IC_{50} = 3.45 \times 10^{-5}$ μM), the standard drug used, was the least potent compound. The potency of the tested compounds can be arranged in descending order as follows: **6**, **8**, **1**, **11**, **9**, **3**, **5**,**7**, **4**, **and 10**.

On neuroblastoma **(GOTO)** and (**NB-1)** cell lines, compound **11** was the most potent compound with ($IC_{50} = 2.53 \times 10^{-5}$ μM) and ($IC_{50} = 2.50 \times 10^{-5}$ μM), respectively whereas, the $IC_{50(s)}$ of **Doxorubicin**, the standard drug used on both cell lines, were

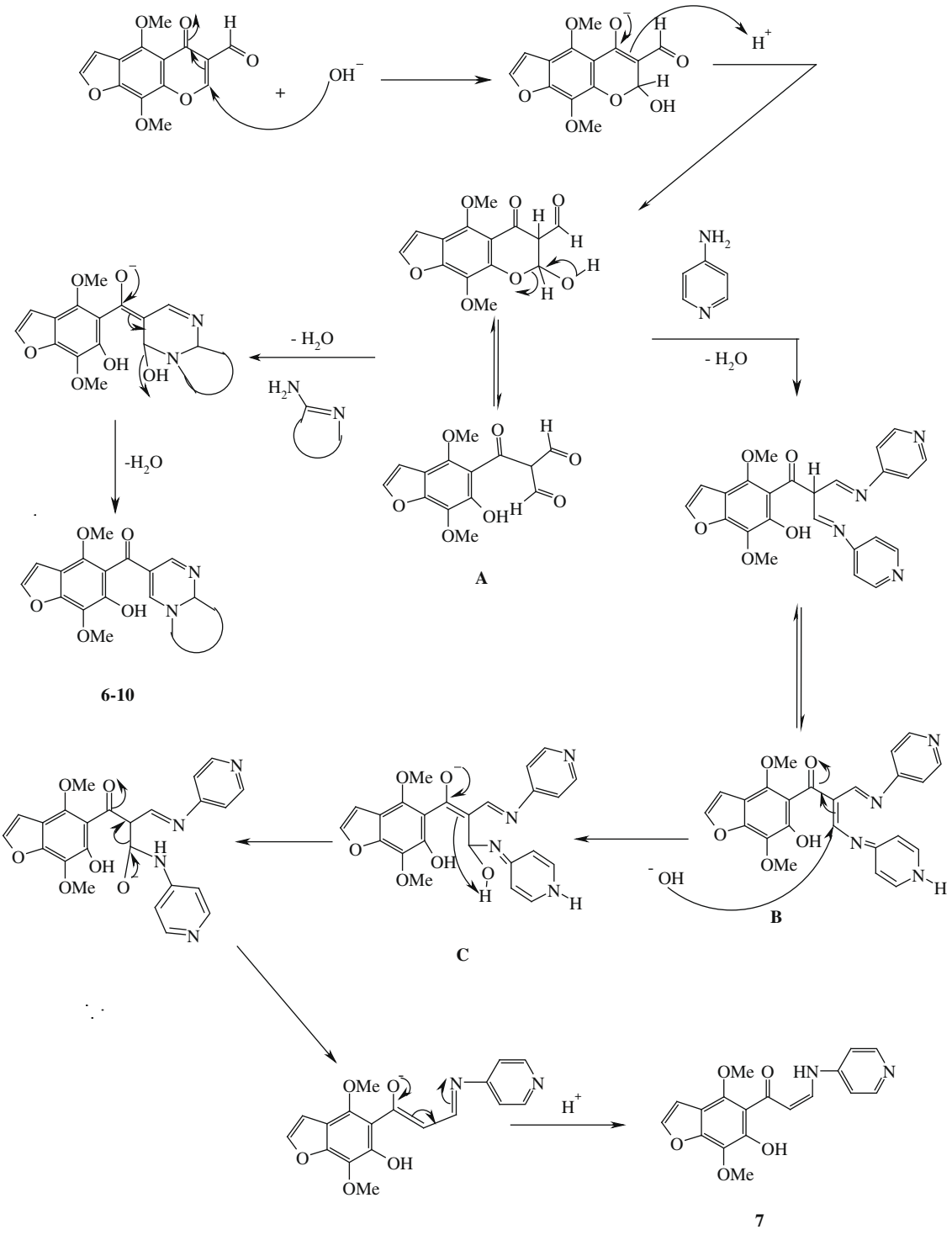


Scheme 3.

(4.73×10^{-3} μM) and (5.15×10^{-3} μM), respectively. By comparing the bioactivity of the synthesized compounds with compound **1**, it was found that compounds **11** and **8** were more potent than compound **1**, whereas compounds **9** and **10** were less potent than **1** while compound **6** and **1** have the same bioactivity, in addition, compounds **4** and **5** were less potent than **3**.

On neuroblastoma **(NB-1**) cell line, comparison of the potency of furochromone **1** with the other tested compounds indicated that formation of compounds **11** and **8** caused increasing in the potency, whereas, compounds **7, 4**, **6** and **9** have lower bioactivity than **1**. On the other hand, compounds **4** and **5** have less potency than compound **3**.

The previous screening of the bioactivity of the tested compounds on the presented panel cell lines indicated that, they were more significantly potent than the comparative standards used. The starting material **1** and the products **4–11** have in common the benzofuran moiety related to a carbonyl group in the 5-position, but the activity varied slightly on the heterocyclic moiety related to the carbonyl group for compounds **4–10** or the side chain in case of compound **11**. Compound **1** is one of the most potent compounds against **SF-268**, **RKOP27**, **K562** and **G361** with slight selectivity toward tumor cell lines over **WI 38** normal cell line. The other starting material **3**, 2-aminonethylbenzimidazole, is one of the most potent compound against **RKOP27** cell line with low selectivity to the tumor cell lines. The imidazodiazepine moiety on the compounds **4** and **5** has positive influence on the activity, where **4** and **5** are among the most active compounds for cell lines **KB**, **SKOV-3** and **NCIH460**. Moreover, compound **5** is one of the most potent for **SF-268** and **RKOP27** cell lines. Compound **4** is relatively selective for tumor cell lines over **WI 38** cell line, while **5** showed no selectivity. Compound **6** with imidazopyrimidine moiety have a great bioactivity against **SK-MEL-28** and **G361**cell lines with selectivity to tumor cell lines. Pyridopyrimidine moiety in compound **7** increases the activity against **SF-268** cell line but with the absence of selectivity over **WI 38** cell line. Whereas, compound **8** with pyrazinopyrimidine moiety improve the activity among the tumorigenic **SF-268** and **NCIH460** cell lines and selectivity over non-tumorigenic **WI 38** cell line. The thiazolopyridine and oxadiazolopyrimidine moieties represent in compounds **9** and **10**, respectively raises the potency toward **NCI H460** cell line with little selectivity over **WI 38** cell line. Besides, compound **9** is the most active compound against **HL60** cell line with selectivity



Scheme 4.

over **WI 38** cell line. On the other hand, compound **10** is one of the most potent compounds against **SKOV-3** cell line with minor selectivity over the normal cell line **WI 38**. For compound **11**, the pyridine nucleus is related to the 5-carbonylbenzofuran derivative through an amino propen-linker, which seems to increase the activity, but at the same time does not conserve the selectivity. Compound **11** gave the most potent activity on these cell lines **RKOP27**, **U937**, **G361**, **GOTO** and **NB-1** cell lines but unfortunately showed no selectivity on **WI 38** cell line with lower or almost the same IC_{50}.

Antiviral activity: HIV inhibitory activity and reverse transcriptase inhibition with therapeutic windows. CEM-SS cells, laboratory-derived virus isolates (including drug-resistant virus isolates), and low-passage clinical virus isolates used in these evaluations were previously described in detail.[26] The inhibitory activities of the tested compounds **1**, **3–11** against HIV were evaluated by microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood mononuclear cells (PBMCs); these assays quantify the ability of a compound to inhibit HIV-induced cell killing or HIV replication. Quantification was performed by the tetrazolium dye XTT as-

Table 1
The cytotoxicity activity of synthesized compounds was determined by using MTT assay according to the Mosmann's method[25] on different human cancer cell lines

Compound #	IC_{50}[a] μM Tumor cell growth inhibition (%)				
	KB	SK OV-3	SF-268	NCI H460	RKOP27
1	3.78×10^{-5}	3.28×10^{-5}	2.55×10^{-5}	2.84×10^{-5}	2.55×10^{-5}
3	2.77×10^{-5}	2.84×10^{-5}	2.84×10^{-5}	3.20×10^{-5}	2.55×10^{-5}
4	2.55×10^{-5}	2.55×10^{-5}	2.77×10^{-5}	2.55×10^{-5}	**2.77** $\times 10^{-5}$
5	2.55×10^{-5}	2.55×10^{-5}	2.55×10^{-5}	2.55×10^{-5}	2.55×10^{-5}
6	2.8×10^{-5}	3.28×10^{-3}	3.00×10^{-5}	2.77×10^{-5}	2.77×10^{-5}
7	3.28×10^{-3}	2.77×10^{-5}	2.55×10^{-5}	2.77×10^{-5}	2.77×10^{-5}
8	2.60×10^{-5}	2.84×10^{-5}	2.55×10^{-5}	2.55×10^{-5}	2.84×10^{-5}
9	2.77×10^{-5}	3.28×10^{-3}	3.3×10^{-5}	2.55×10^{-5}	2.8×10^{-5}
10	2.77×10^{-5}	2.55×10^{-5}	2.8×10^{-5}	2.55×10^{-5}	2.60×10^{-5}
11	4.5×10^{-5}	3.20×10^{-5}	2.84×10^{-5}	2.84×10^{-5}	2.55×10^{-5}
Fluorouracil	4.46×10^{-3}				
Doxorubicin		4.16×10^{-3}			
Cytarabine			7.68×10^{-3}		
Gemcitabine HCl				2.13×10^{-3}	
Capecitabine					4.33×10^{-3}

Table 2
The cytotoxicity activity of synthesized compounds was determined by using MTT assay according to the Mosmann's method[25] on different human cancer cell lines

Compound #	IC_{50}[a] μM Tumor cell growth inhibition (%)							
	Leukaemia			Melanoma		Neuroblastoma		Normal cells
	HL60	U937	K562	G361	SK-MEL-28	GOTO	NB-1	WI 38
1	3.13×10^{-5}	2.91×10^{-5}	2.58×10^{-5}	2.63×10^{-5}	2.66×10^{-5}	2.78×10^{-5}	3.16×10^{-5}	3.21×10^{-5}
3	2.91×10^{-5}	2.98×10^{-5}	3.13×10^{-5}	3.05×10^{-5}	3.01×10^{-5}	2.63×10^{-5}	2.78×10^{-5}	2.69×10^{-5}
4	3.25×10^{-5}	3.33×10^{-5}	3.38×10^{-5}	3.16×10^{-5}	3.21×10^{-5}	3.29×10^{-5}	3.33×10^{-5}	3.33×10^{-5}
5	3.05×10^{-5}	3.01×10^{-5}	2.98×10^{-5}	3.13×10^{-5}	3.05×10^{-5}	2.91×10^{-5}	2.91×10^{-5}	2.84×10^{-5}
6	2.60×10^{-5}	2.72×10^{-5}	2.69×10^{-5}	2.63×10^{-5}	2.53×10^{-5}	2.78×10^{-5}	3.97×10^{-5}	4.72×10^{-5}
7	2.75×10^{-5}	2.98×10^{-5}	4.46×10^{-5}	4.81×10^{-5}	3.05×10^{-5}	3.29×10^{-5}	3.21×10^{-5}	2.55×10^{-5}
8	2.66×10^{-5}	2.84×10^{-5}	3.21×10^{-5}	2.98×10^{-5}	2.60×10^{-5}	2.58×10^{-5}	2.78×10^{-5}	3.73×10^{-5}
9	2.55×10^{-5}	2.84×10^{-5}	4.46×10^{-5}	3.84×10^{-5}	2.84×10^{-5}	2.81×10^{-5}	4.31×10^{-5}	2.84×10^{-5}
10	2.81×10^{-5}	3.29×10^{-5}	2.84×10^{-5}	2.87×10^{-5}	3.38×10^{-5}	2.84×10^{-5}	3.25×10^{-5}	2.81×10^{-5}
11	2.63×10^{-5}	2.63×10^{-5}	2.63×10^{-5}	2.63×10^{-5}	2.81×10^{-5}	2.53×10^{-5}	2.50×10^{-5}	2.53×10^{-5}
Doxorubicin	1.13×10^{-3}	4.45×10^{-3}	6.66×10^{-3}					
Aldesleukin				6.66×10^{-3}	3.45×10^{-3}			
Doxorubicin						4.73×10^{-3}	5.15×10^{-3}	

say (CEM-SS, 174 × CEM, MT2, and AA5 cell-based assays), which is metabolized to a colored formazan product by viable cells. Antiviral and toxicity data were reported as the quantity of drug required to inhibit virus-induced cell killing or virus production by 50% (EC_{50}). The compounds **1**, **3–11** were tested for RT inhibitory activity against purified recombinant HIV-1 RT using the cell-free Quan-T-RT assay system (Amersham Corp., Arlington Heights, IL), which utilizes the scintillation proximity assay (SPA) principle.[27,28] $IC_{50[RT]}$ values (concentration at which the compound inhibits recombinant RT by 50%) were calculated by comparing the measurements to untreated sample.

Results of HIV and HIV-RT inhibitory activity of the tested compounds and standard were evaluated (c.f. Table 3). For comparison, **Atevirdine** was used as standard drug. The EC_{50} values listed in Table 3 showed that, all of tested compounds were more potent than **Atevirdine** ($EC_{50} = 10 \times 10^{-4}$ μM). Only the imidazopyrimidinyl derivative **6** ($EC_{50} = 2 \times 10^{-5}$ μM) had much better therapeutic index (TI = 1,140,000) than the standard (TI = 100,000). Studying the bioactivity of new synthesized compounds with respect to furochromone **1**, indicated that compound **11** had higher potency, whereas compound **6** possessed the same bioactivity as compound **1** with wider therapeutic index. On the other hand, compounds **7**, **9**, **10**, **8** and **4** have less bioactivity with respect to furochromone **1**.

Screening of HIV-1 reverse transcriptase inhibition of the tested compounds showed that the oxadiazolopyrimidinyl derivative **10** was the most potent compound with striking IC_{50} (3×10^{-4} μM), followed by compound **11** (IC_{50} = 4.8 μM), then compound **3** (IC_{50} = 5.1 μM), which was more potent than compound **4** (IC_{50} = 9.44 μM). These compounds were more potent than **Atevirdine** (IC_{50} = 10 μM). On the other hand, compounds **1**

Table 3
The HIV inhibitory activity and reverse transcriptase inhibition with therapeutic windows of the tested compounds and standard

Compounds #	EC50*/μM[a]	IC50*/μM[b]	Therapeutic index[c]
1	2 × 10-5	17.53	32,567
3	1×10^{-5}	5.1	1755
4	35×10^{-3}	9.44	21,678
5	6×10^{-5}	47.55	23,458
6	2×10^{-5}	25.66	1,140,000
7	3×10^{-5}	16.3	23,498
8	9×10^{-5}	14.6	20,000
9	4×10^{-5}	15.26	22,567
10	4×10^{-5}	3×10^{-4}	7689
11	1×10^{-5}	4.8	1263
Atevirdine	10×10^{-4}	10	100,000

[a] Compound concentration required to inhibit the virus induced cell killing by 50%.
[b] Compound concentration required to achieve 50% inhibition of recombinant HIV-1 RT activity.
[c] Therapeutic index is the toxic dose of a drug for 50% of the population (TD_{50}) divided by the minimum effective dose for 50% of the population (ED_{50}).
* EC_{50} and IC_{50} values were estimated by logistic regression analysis. One way ANOVA ($P < 0.01$) was used to test treatment difference in EC_{50} and IC_{50}. After significant factor by ANOVA, individual group differences were analyzed using Holm-Sidak's procedure for multiple comparisons versus control.

(IC$_{50}$ = 17.53 μM), **8** (IC$_{50}$ = 14.6 μM), **9** (IC$_{50}$ = 15.26 μM), **7** (IC$_{50}$ = 16.3 μM), **6** (IC$_{50}$ = 25.66 μM) and **5** (IC$_{50}$ = 47.55 μM), respectively, showed significant activity but less than the standard.

Hepatitis C Virus (HCV) NS3-4A protease inhibitor activity. Hepatitis C virus (HCV) NS3-4A protease inhibitor activity of the compounds **1**, **3–11** was tested. Cells were identical in sequence to the I_{377}neo/NS3-3′/wt replicon described by Lohmann et al.[29] Determination of 50% inhibitory concentration (IC$_{50}$), 90% inhibitory concentration (IC$_{90}$), and 50% cytotoxic concentration (CC$_{50}$) of all the tested compounds in HCV replicon cells was performed using a quantitative RT-PCR (QRT-PCR) assay.[30,31] The cytotoxicity of the tested compounds was measured under the same experimental settings using a tetrazolium (MTS)-based cell viability assay (Promega, Madison, WI) for comparison was used as standard drug. The results of **HCV NS3-4A** protease inhibitor activity and the cytotoxic activity of the tested compounds and standard were listed (c.f. Table 4).

Screening of the 90% inhibitory concentration (IC$_{90}$) values revealed that compounds **1** (IC$_{90}$ = 0.796 ± 0.08 μM), **4** (IC$_{90}$ = 0.955 ± 0.08 μM), **9** (IC$_{90}$ = 1.12 ± 0.12 μM), **6** (IC$_{90}$ = 1.32 ± 0.13 μM), **3**(IC$_{90}$ = 2.12 ± 0.12 μM), and **11**(IC$_{90}$ = 2.31 ± 0.2 μM), respectively, showed significant activity but less active than **VX-95**. Whereas, compounds **5**, **7**, **8** and **10** were inactive.

Regarding to the cytotoxicity on hepatocyte cell lines, the standard drug used was also the most potent compound (CC$_{50}$ =90 ± 1.2 μM). Compound **9** (CC$_{50}$ = 127 ± 9 μM) had the most significant potency among the synthesized compounds followed by compounds **1** and **6** with the same bioactivity (CC$_{50}$ = 234 ± 6 μM), then compounds **4** (CC$_{50}$ = 256 ± 13 μM), **3** (CC$_{50}$ = 345 ± 12) and **11** (CC$_{50}$ = 435 ± 15 μM). On the other hand, the bioactivity of compounds **7**, **8** and **10** almost decayed. Studying the therapeutic index of the tested compound, revealed that none of the tested compound has wider therapeutic index than **VX-950**.

The median lethal dose ***LD$_{50}$*** of the tested compounds was determined according to the procedure described by Lorke.[32–34] The results of the LD$_{50}$ in mg/kg of the synthesized compounds were listed (c.f. Table 5). Screening the median lethal dose (LD$_{50}$) of the tested compounds **1**, **3–11** showed that, all the compounds were found to have higher LD$_{50}$ than compound **1** (LD$_{50}$ = 113.29 ± 1 mg/kg). These results indicated that the heteromoieties included into benzofuranyl skeleton decreased the toxicity of the starting material **1**.

In conclusion, the reaction of furochromone **1** or **2** with heterocyclic amines afforded antitumor and antiviral active agents via ring opening reaction. The cytotoxicity studies of compounds **1**, **3–11** against 12 human cancer and the non-tumorigenic **WI 38** cell lines showed that, the synthesized compounds possess high potency against all cell lines and the hetero-moieties included into bezofuranylcarbonyl skeleton via ROR, mostly have positive effect on the activity and selectivity.

The HIV inhibitory activity of the tested compounds, **1**, **3–11**, indicated that all the compounds were more active than **Atevirdine** and compound **6** having imidazopyrimidine as heterocyclic moiety has 20 times active as **Atevirdine** with higher therapeutic index. Testing of the HIV-RT inhibitory activity of compounds, showed that compound **10** was the most potent one but with lower therapeutic index than the standard.

The evaluation of hepatitis C virus (HCV) NS3-4A protease inhibitor activities showed that compound **1** was the most active compound. Only compound **9** having the thiazolopyridine as heteromoiety is nearly active as **VX-950** with regarding to the cytotoxicity on hepatocyte cell lines. Unfortunately, none of the tested compounds was more potent and had wider therapeutic index than **VX-950**, the standard drug used.

All the synthesized derivatives can serve as lead compounds for further investigations or may constitute a basis of new class of antitumor and antiviral active agents.

*(11H-Benzo[4,5]imidazo[1,2-a][1,4]diazepin-4-yl)(6-hydroxy-4,7-dimethoxy-benzofuran-5-yl)methanone (**4**).* Yield: 83%, mp 315–317 °C. ^{1}H NMR (500 MHz, DMSO-d_6): 3.94(s, 3H, CH$_3$), 4.00(s, 3H, CH$_3$), 7.14(d, 1H, *J* = 1.5 Hz, H3′ benzofuran), 7.47 (m, 2H, H8 and H9 benzimidazodiazepine), 7.56(d, 1H, *J* = 1.5 Hz, H2′ benzofuran), 7.72(m, 4H, H1, H3, H7 and H10 diazepinobenzimidazole), 7.91(s, 1H, H5 benzimidazodiazepine), 9.23(br, NH, D$_2$O exchange-

Table 5
The median lethal dose of the tested

Compound no.	(LD$_{50}$[a] ± SD[*])[**] mg/kg
1	113.29 ± 1
3	146.6 ± 1
4	165.65 ± 1
5	121.16 ± 1
6	177.14 ± 1
7	124.67 ± 1
8	150.09 ± 1
9	133.14 ± 1
10	125.98 ± 1
11	152.13 ± 1

[a] Dose required to kill half the members of the tested population.
[*] Data expressed as means ± *SD* for three independent experiments.
[**] The median lethal dose of the tested compounds was determined according to the procedure described by Lorke.

Table 4
HCV NS3-4A protease inhibitory activity and the cytotoxic activity of the tested compounds and standard

Compound no.	IC$_{50}$[a] (± SD[*])μM after 48 h	IC$_{90}$[b] (± SD[*])μM after 48 h	CC50[c] (± SD[*])μM	Therapeutic index[d]
1	0.421 ± 0.03	0.796 ± 0.08	234 ± 5	555.81948
3	0.876 ± 0.09	2.12 ± 0.12	451 ± 12	514.84018
4	0.444 ± 0.04	0.955 ± 0.08	256 ± 13	576.57658
5	>100	—	—	—
6	0.564 ± 0.04	1.32 ± 0.13	234 ± 6	414.89362
7	>100	—	—	—
8	>100	—	—	—
9	0.342 ± 0.03	1.12 ± 0.12	127 ± 9	371.34503
10	>100	—	—	—
11	0.986 ± 0.08	2.31 ± 0.2	435 ± 15	441.17647
VX-950 [e]	0.20 ± 0.0001	0.45 ± 0.0003	90 ± 1.2	1200

[a] Compound concentration required to achieve 50% inhibition of HCV NS3-4A protease activity.
[b] Compound concentration required to achieve 90% inhibition of HCV NS3-4A protease activity.
[c] Compound concentration required to reduce the cell viability by 50% as determined by MTT method.
[d] Therapeutic index is the toxic dose of a drug for 50% of the population (TD$_{50}$) divided by the minimum effective dose for 50% of the population (ED$_{50}$).
[e] The standard used in comparison.
[*] Data expressed as means ± SD for three independent experiments.

able), 12.75(s, 1H, OH, D_2O exchangeable), IR (cm^{-1}): 3503.75 (OH), 3415.73 (NH), 3064.93(CH, aromatic), 2970.36 (CH, aliphatic), 3164.74–2655.42 (intermolecular H bonded OH and NH), 1641.1(C=O), 1608.45 (C=N), 1540.45(C=C). MS: *m/z* 403 (M^+, 45%).220 (m_1, 100%), 183 (m_2, 93%), Anal. Calcd for $C_{22}H_{17}N_3O_5$: C, 65.50; H, 4.25; N, 10.42. Found: C, 65.43; H, 4.29; N, 10.40.

*(11H-Benzo[4,5]imidazo[1,2-a][1,4]diazepin-4-yl)(6-hydroxy-4-methoxy-benzofuran-5-yl)methanone (**5**). Yield: 63%, mp 323–325 °C.* ^1H NMR (500 MHz, DMSO-d_6): 3.96(s, 3H, CH_3), 7.16(s, 1H, *J* = 1.5 Hz, H3′ benzofuran), 7.20 (m, 3H, H7′ benzofuran, H8 and H9 benzimidazodiazepine), 7.48(d, 1H, *J* = 1.5 Hz, H2′ benzofuran),7.73(m, 4H, H1, H3, H7 and H10 diazepinobenzimidazole), 8.1(s, 1H, H5 benzimidazodiazepine), 9.23(br, NH, D_2O exchangeable), 12.752(s, 1H, OH, D_2O exchangeable), IR (cm^{-1}): 3520.35(OH), 3404.21(NH), 3034.88(CH, aromatic), 2974.50(CH, aliphatic), 1639.73(C=O), 1608.33 (C=N). MS: *m/z* 373(M^+, 100%). Anal. Calcd for $C_{21}H_{15}N_3O_4$: C, 67.56; H, 4.05; N, 11.25. Found: C, 67.53; H, 4.12; N, 11.11

*(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)(benzo[4,5]imidazo[1,2-a]pyrimidin-3-yl)methanone (**6**).* Yield: 65%, mp >330 °C. ^1H NMR (500 MHz, DMSO-d_6) 3.84(s, 3H, CH_3), 3.93(s, 3H, CH_3), 7.15 (m 3H, H3′ benzofuran, H7 and H8 benzimidazopyrimidine), 7.64 (m, 1H, H9 benzimidazopyrimidine), 7.70(d, 1H, *J* = 1.5 Hz, H2′ benzofuran), 8.28(m, 1H, H6 benzimidazopyrimidine), 8.51(s, 1H, H4 benzimidazopyrimidine), 8.87(s, 1H, H2 benzimidazopyrimidine), 13.02(s, 1H, OH, D_2O exchangeable). IR(cm^{-1}): 3571.52(OH), 3050.33(CH, aromatic), 2975.33(CH, aliphatic), 3200.74–2600.55(intermolecular H bonded OH), 1642.09(C=O), 1606.8(C=N), 1537.35(C=C). MS: *m/z* 389(M^+, 10.5%), 221(m_1, 77%), 169(m_2, 100%). Anal. Calcd for $C_{21}H_{15}N_3O_5$: C, 64.78; H, 3.88; N, 10.79. Found: C, 64.58; H, 3.91; N, 10.83.

*(6-hydroxy-4,7-dimethoxybenzofuran-5-yl)(9aH-pyrido[1,2-a]pyrimidin-3-yl)methanone (**7**).* Yield: 77%, mp >340 °C. ^1H NMR (500 MHz, DMSO-d_6): 3.88(s, 3H, CH_3), 3.96(s, 3H, CH_3), 4.59(s, 1H, H9a pyridopyrimidine), 6.17(m, 1H, H9 pyridopyrimidine), 6.45(m, 1H, H8 pyridopyrimidine), 6.62(m, 1H, H6 pyrimidobenzimidazole), 6.96(m, 1H, H7 pyridopyrimidine), 7.17(d, 1H, *J* = 1.5 Hz, H3′ benzofuran), 7.69(d, 1H, H2′ benzofuran), 7.79(m, 1H, H2 pyrimidobenzimidazole), 7.95(s, 1H, H4 pyrimidobenzimidazole), 12.75(s, 1H, OH, D_2O exchangeable). IR (cm^{-1}): 3532.67(OH), 3035.18(CH, aromatic), 2972.10(CH, aliphatic), 3200–2600 (intermolecular H bonded OH), 1639.2, (C=O), 1607.38(C=N), 1547.21(C=C). MS: *m/z* 352(M^+, 80.12%), 220 (m_1, 95%), 132(m_2, 100%). Anal. Calcd for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95. Found: C, 64.69; H, 4.62; N, 7.85.

*(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)(8H-pyrazino[1,2-a]pyrimidin-3-yl)methanone (**8**).* Yield: 75%, mp 215–217 °C (dec.). ^1H NMR (500 MHz, DMSO-d_6): 2.80(s, 1H, NH, D_2O exchangeable), 3.85(s, 3H, CH_3), 3.92(s, 3H, CH_3), 5.95(s, 1H, H9 pyrzinopyrimidine), 6.22(m, 2H, H6 and H7 pyrzinopyrimidine),7.09(d, 1H, *J* = 1.5 Hz,, H3′ benzofuran), 7.60(d, 1H, *J* = 1.5 Hz, H2′ benzofuran), 7.68(s, 1H, H2 pyrzinopyrimidine), 7.82(s, 1H, H4 pyrzinopyrimidine), 13.09(s, 1H, OH, D_2O exchangeable). IR (cm^{-1}): 3574.37(OH), 3241.64(NH), 1645.34(C=O), 1597.84(C=N). MS: *m/z* 353 (M^+, 65.12%), 220(m_1, 87%), 132(m_1, 100%). Anal. Calcd for $C_{18}H_{15}N_3O_5$**:** C, 61.19; H, 4.28; N, 11.89. Found: C, 61.22; H, 4.17; N, 11.86.

*(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)(8aH-thiazolo[3,2-a]pyrimidine-6-yl)methanone (**9**).* Yield: 75%, mp >340 °C. ^1H NMR (500 MHz, DMSO-d_6): 3.88(s, 3H, CH_3), 3.92(s, 3H, CH_3), 4.52(s, 1H, H8a thiazolopyrimidine), 5.87(d, *J* = 6.5 Hz, 1H, H2 thiazolopyrimidine), 6.52(d, *J* = 6.5 Hz, 1H, H3 thiazolopyrimidine), 7.16(d, 1H, *J* = 1.5 Hz, H3′ benzofuran), 7.53(m, 2H, H7 thiazolopyrimidine), 7.61(d, 1H, *J* = 1.5 Hz, H2′ benzofuran), 7.81(s, 1H, H5 thiazolopyrimidine), 13.23(s, 1H, OH, D_2O exchangeable). IR (cm^{-1}): 3513.00(OH), 3350.6(NH), 1655.65(C=O), 1611.12(C=N). MS: *m/z* 358 (M^+, 98%), 220(m_1, 100%), 138(m_2, 81%), Anal. Calcd for $C_{17}H_{14}N_2O_5S$**:** C, 56.98; H, 3.94; N, 7.82; S, 8.95. Found**:** C, 56.91; H, 3.88; N, 7.89; S, 8.95.

*(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)(2-methyl-3aH-[1,2,4]-oxadiazolo[2,3-a]pyrimidin-6-yl)methanone (**10**).* Yield: 75%, mp 225–227 °C. ^1H NMR (500 MHz, DMSO-d_6): 1.52(s, 3H, CH_3, oxadiazolopyrimidine), 3.85(s, 3H, OCH_3), 3.92(s, 3H, OCH_3), 3.75(s, 1H, H3a, oxadiazolopyrimidine), 6.23(s, 1H, H7 oxadiazolopyrimidine), 7.16(d, 1H, *J* = 1.5 Hz, H3′ benzofuran), 7.61(d, 1H, *J* = 1.5 Hz, H2′ benzofuran), 7.81(s, 1H, H5 oxadiazolopyrimidine), 12.65(s, 1H, OH, D_2O exchangeable). IR (cm^{-1}): 3402.31(br, OH and NH), 1639.34(C=O), 1617.81(C=N). MS: *m/z* 335(M^+, 65.12%), 132 (m_1, 100%). Anal. Calcd for $C_{17}H_{15}N_3O_6$**:** C, 57.14; H, 4.23; N, 11.76. Found: C, 57.22; H, 4.17; N, 11.86.

*(E)-1-(6-Hydroxy-4,7-dimethoxybenzofuran-5-yl)-3-(pyridin-4-ylamino)prop-2- en-1-one (**11**).* Yield: 60%, mp 295–297 °C. ^1H NMR (500 MHz, DMSO-d_6): 3.85(s, 3H, OCH_3), 3.93(s, 3H, OCH_3), 6.42(d, 1H, *J* = 18.5 Hz, H2″ propenone), 7.2(d, 1H, *J* = 2 Hz, H3′ benzofuran), 7.42(d, 2H, *J* = 7.5 Hz, H3 and H5 pyridine), 7.53(d, 1H, *J* = 18.5 Hz, H3″ propenone), 7.76(d, 1H, *J* = 2 Hz, H2′ benzofuran), 8.30(d, 2H, *J* = 7.5 Hz, H2 and H6 pyridine), 10.56(s, 1H, NH, D_2O exchangeable), 12.96(s, 1H, OH, D_2O exchangeable). IR (cm^{-1}): 3513.00 (OH), 3350.62(NH), 1655.65(C=O), 1611.12(C=N). MS: *m/z* 340 (M^+, 55%), 220(m_1, 100%). Anal. Calcd for $C_{18}H_{16}N_2O_5$**:** C, 63.52; H, 4.74; N, 8.23. Found: C, 63.55; H, 4.79; N, 8.12.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.069.

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